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Advancing Nucleic Acid TechnologySM

Oligo Design Across the Mouse Genome

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03/11/2009


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Fluorescence-Quenched Probes

5'-FAM - CAAGTTTGACCAAGTCACAACGGC-BHQ1-3'



|||||
...CACGTTGTTCAAACCTGGTTCAGTGTTGCCGATTAGA...



“the combination of the hybridization-specific probes and PCR amplification provided combined sensitivity, speed, specificity, and simplicity.”

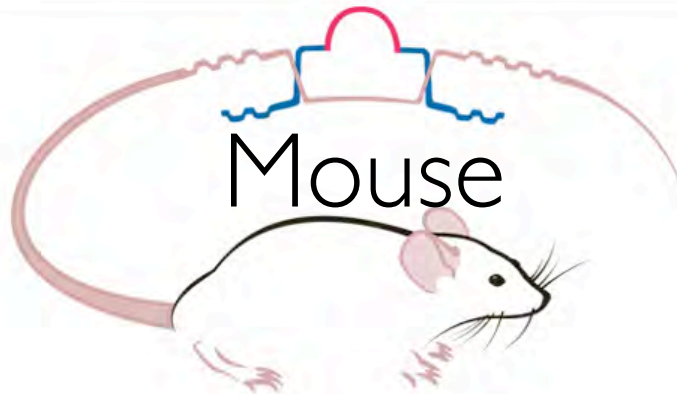
-Morrison, et al.

Morrison L. E., et al. (1989) Solution-phase detection of polynucleotides using interacting fluorescent labels and competitive hybridization. *Analytical biochemistry* 183(2): 231-244.

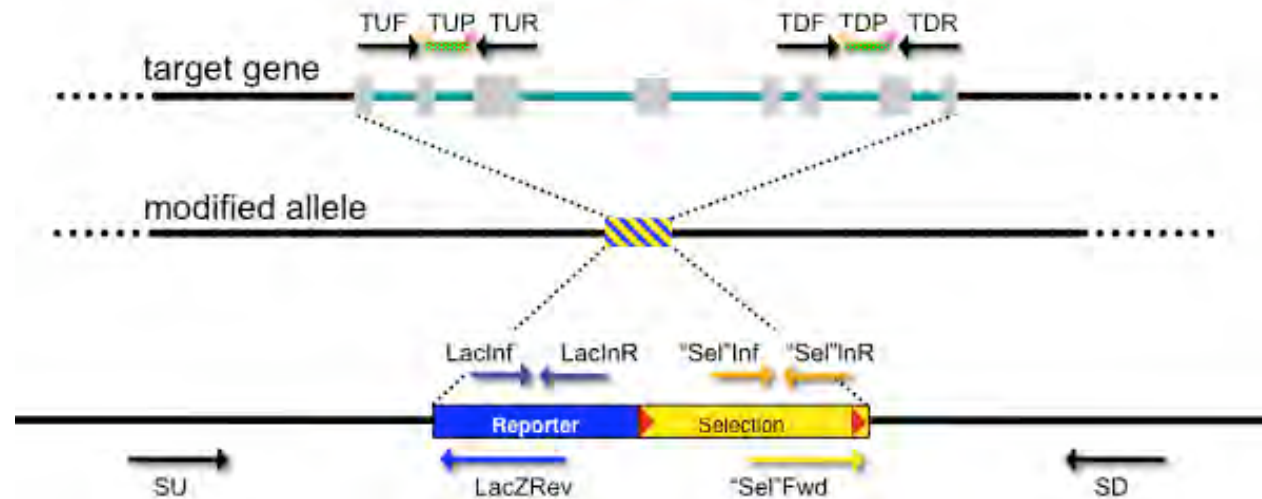


Knockout

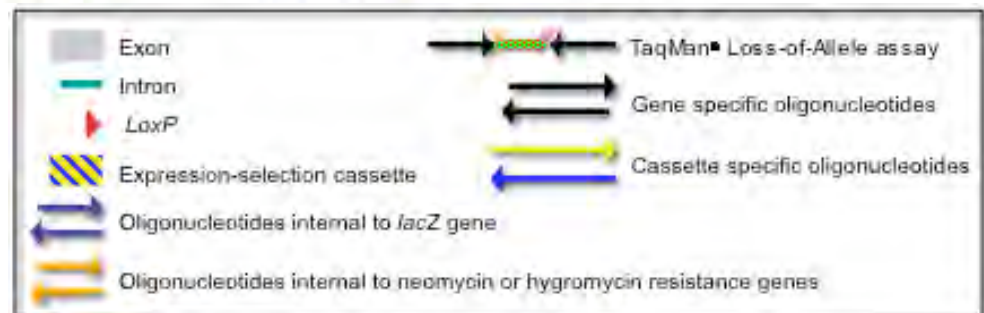
Project



VelociGene's KOMP Allele Genotyping Strategies

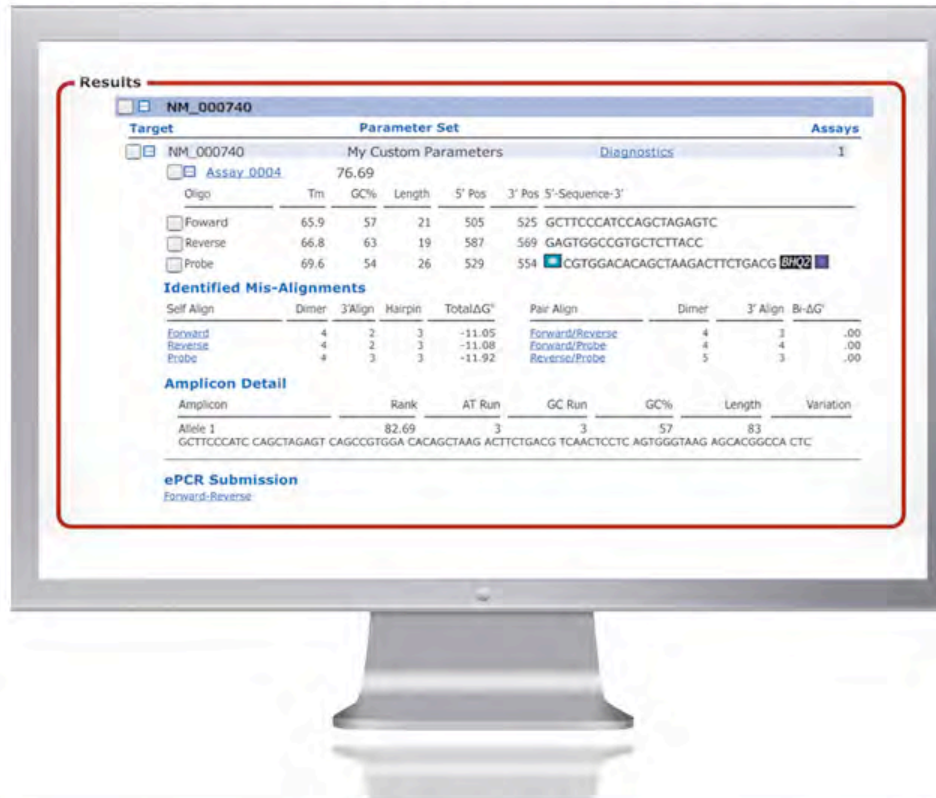


Valenzuela, D. M., et al. (2003). High-throughput engineering of the mouse genome coupled with high-resolution expression analysis. *Nat Biotechnol* 21, 652-659.



REALTIMEDESIGN™

www.qpcrdesign.com



High Throughput:
Ten different assays designed simultaneously.

Adjustable Parameters
Custom values to make default for future.

Design Archives:
History stores 100 different design sessions.

Free to use,
entirely through the web.

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TECHNOLOGIES**

Oligo Design Considerations

- Unique identification of the target sequence

CATAATCGTTGCTATTAACCACTTTATGAAAATCTAACTGGACATGGTTTTTCACTACTTTTAGCAGGTGG

- Binding stability that is tuned to the temperature profile of the assay



- Signal presence of the amplifying target



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Sequence Designs to Date

86.5% of all assays designed using three default parameter sets.

The Most Restrictive parameters yield a 96.7% success rate.

The Less Restrictive parameters yield a 96.4% success rate.

The Least Restrictive parameters yield a 94.4% success rate.

Assays designed on a custom basis yield a 83.6% success rate.

> 5,000 assays synthesized.

> 4,500 have been tested.

~ 93% success rate for new designs.

~ 80% success rate for re-designs.

Difficult targets demand BHQplus™



Design Archives & Custom Parameters

Parameter Sets

Changes made here affect only your personal parameter sets.

Personal Parameter Set	Express/Batch Order	Use	Custom Default
Less Restrictive v2.0	▼	<input checked="" type="checkbox"/>	<input type="radio"/>
Most Restrictive v2.0	▲ ▼	<input checked="" type="checkbox"/>	<input checked="" type="radio"/>
Least Restrictive v2.0	▲	<input checked="" type="checkbox"/>	<input type="radio"/>
My Custom Parameters		<input type="checkbox"/>	<input type="radio"/>
Most Restrictive v1.0		<input type="checkbox"/>	<input type="radio"/>
Less Restrictive v1.0		<input type="checkbox"/>	<input type="radio"/>
Least Restrictive v1.0		<input type="checkbox"/>	<input type="radio"/>

Parameters for TaqMan®-Quantitative PCR-Custom

primerset assay Forward Reverse TaqMan Probe

Parameter	Settings			Weight	Use
	Min	Ideal	Max		
Amplicon Length	60	80	100	3	<input type="checkbox"/>
Amplicon GC Percent	40.0	50.0	60.0	5	<input checked="" type="checkbox"/>
Amplicon ACT Mono Run Length	Ideal	Max		0	<input checked="" type="checkbox"/>
Amplicon G Mono Run Length	Ideal	Max		0	<input checked="" type="checkbox"/>
Forward/Reverse Pair - Duplex	Ideal	Max		2	<input checked="" type="checkbox"/>
Forward/Reverse Pair - Match Count	Ideal	Max		2	<input checked="" type="checkbox"/>
Forward/Reverse	Ideal	Max	Analysis Len		<input checked="" type="checkbox"/>

Current Design Runs

Select	Name/Description	Design Date	#Seq	#w/ Assays	
<input type="checkbox"/>	BG536849, CX698698, ...	02/02/08 03:24 PM	10	10	Details
<input type="checkbox"/>	AY859741, NM_0010035...	02/02/08 03:19 PM	10	9	Details
<input type="checkbox"/>	NM_000740	01/24/08 09:33 AM	1	1	Details
<input type="checkbox"/>	AY289206	01/16/08 11:22 AM	1	1	Details
<input type="checkbox"/>	UGT2B15 A/G, UGT2B15...	12/17/07 01:22 PM	10	8	Details
<input type="checkbox"/>	TBXAS1 A/G, PTGIS G/...	12/17/07 01:17 PM	10	9	Details
<input type="checkbox"/>	CYP3A5 A/-	12/14/07 10:00 AM	1	1	Details
<input type="checkbox"/>	DPYD - /ATGA	12/14/07 09:56 AM	1	1	Details
<input type="checkbox"/>	NAT1 GG/CC	12/14/07 09:55 AM	1	1	Details

1 2 3 4 5 6

Archive

Execute

Assay Design - Gsdma2

10517TD

Target	Parameter Set	Assays
10517TD	1-KOMP Most Restrictive	Diagnostics 1

Mus musculus genome

[Hide Alignments](#) [Show all hits in MapViewer](#)

Amplicon1 Hits: 1

Chr	Location	Gene	obs.size	exp.size	L m/g	R m/g
11	98,519,097..98,519,185 (+)	Gsdma2	89	89	0/0	0/0

Primers: AGCCTTCCCGGCCCTTACTG <L R> CAGTCAGGAGGCAGAGGCAGT

Genome: AGCCTTCCCGGCCCTTACTG ... CAGTCAGGAGGCAGAGGCAGT

Allele 1
 AGCCTTCCCGGCCCTTACTGCTGCTGTGTCTGAAACACTCCAAGATGGGTGGCAATTCACCTTTAATCCAGCA GTCAGGAGGC
 AGAGGCAGT

ePCR Submission

[Forward-Reverse](#)

Confirm assay specificity *in silico*



Assay Design - Bmp8a

Mus musculus genome

Amplicon1 Hits: 4

Chr	Location	Gene	obs.size	exp.size	L m/g	R m/g
4	122,793,763..122,793,837 (+)	Bmp8b	75	75	0/0	0/0
Primers: GAAGACCAAGGGCGTGAAG <L R> GACTTCGGCCTGGGCATCTT Genome: GAAGACCAAGGGCGTGAAG...GACTTCGGCCTGGGCATCTT						
4	122,793,763..122,793,837 (+)	Oxct2b	75	75	0/0	0/0
Primers: GAAGACCAAGGGCGTGAAG <L R> GACTTCGGCCTGGGCATCTT Genome: GAAGACCAAGGGCGTGAAG...GACTTCGGCCTGGGCATCTT						
4	123,000,526..123,000,600 (-)	Bmp8a	75	75	0/0	0/0

Genomic context

chromosome: 4; Locations: 4 D2.2; 4 57.4 cM [See Bmp8a in MapViewer](#)

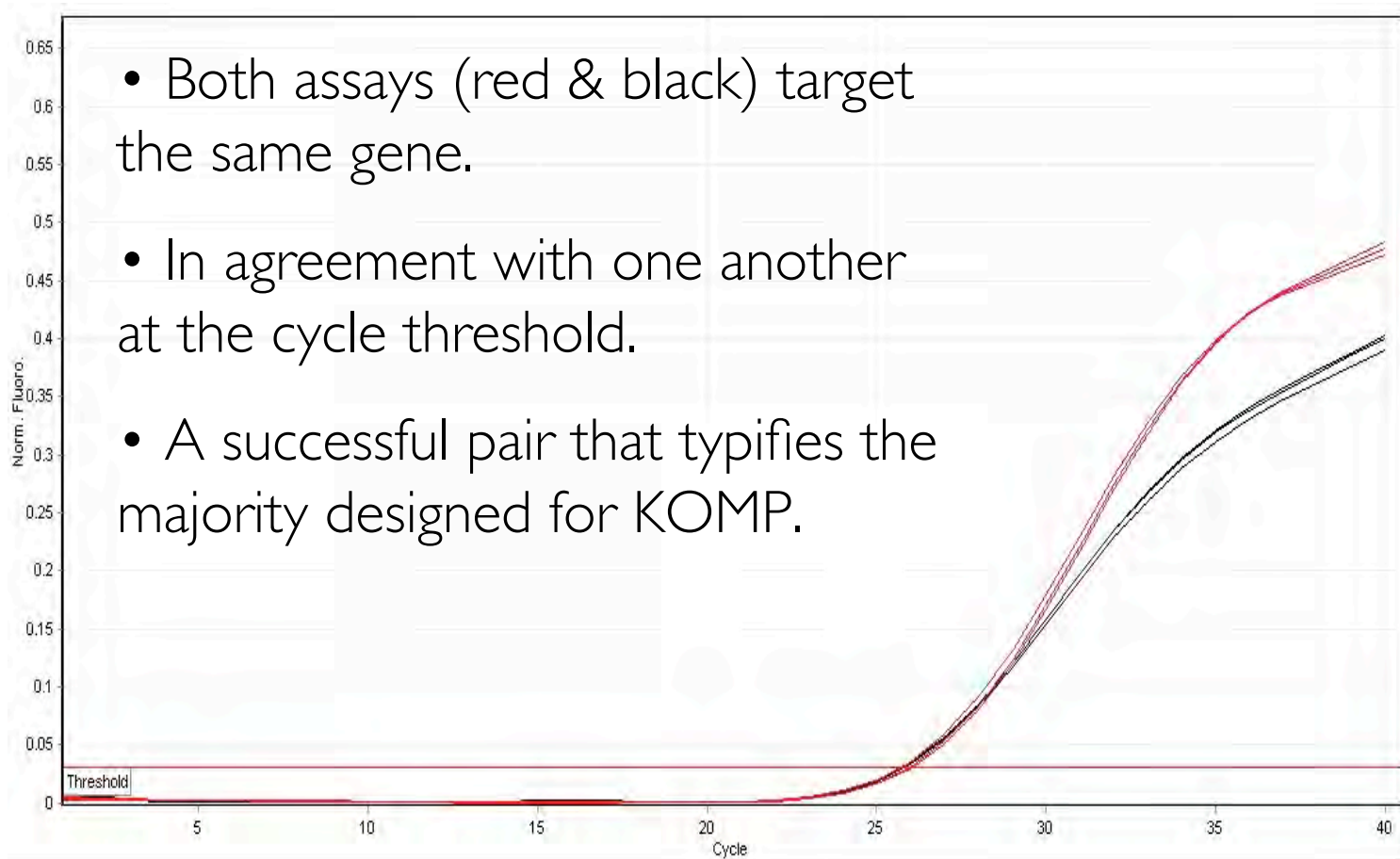
OTTHUSG00+++ [122952117] Bmp8a D830031N0+++ [123387270] OTTHUSG00+++
 Pabpc4 Oxct2a Hecf1

Confirm assay specificity *in silico*



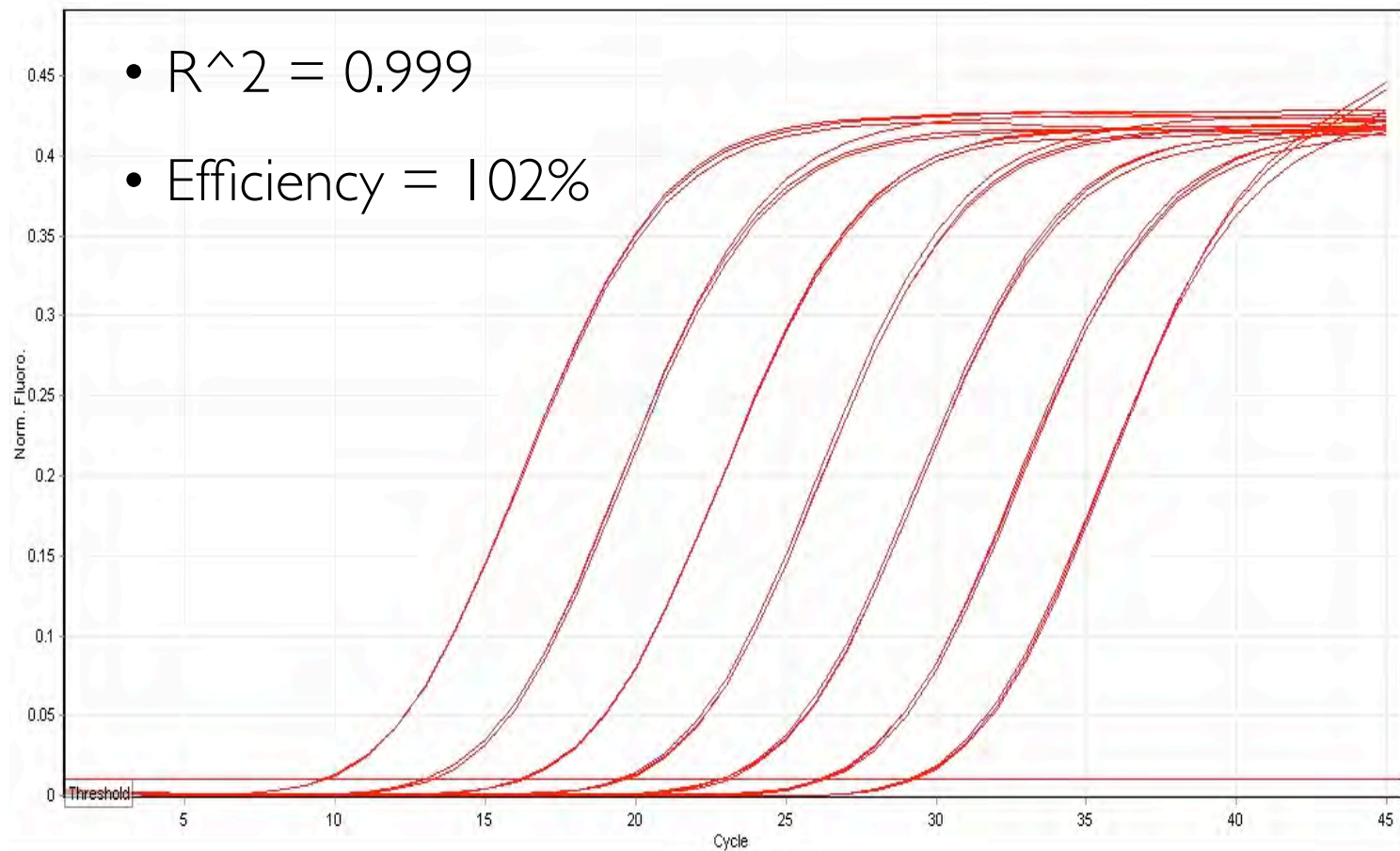
Amplification Performance - Gsdma2

- Both assays (red & black) target the same gene.
- In agreement with one another at the cycle threshold.
- A successful pair that typifies the majority designed for KOMP.



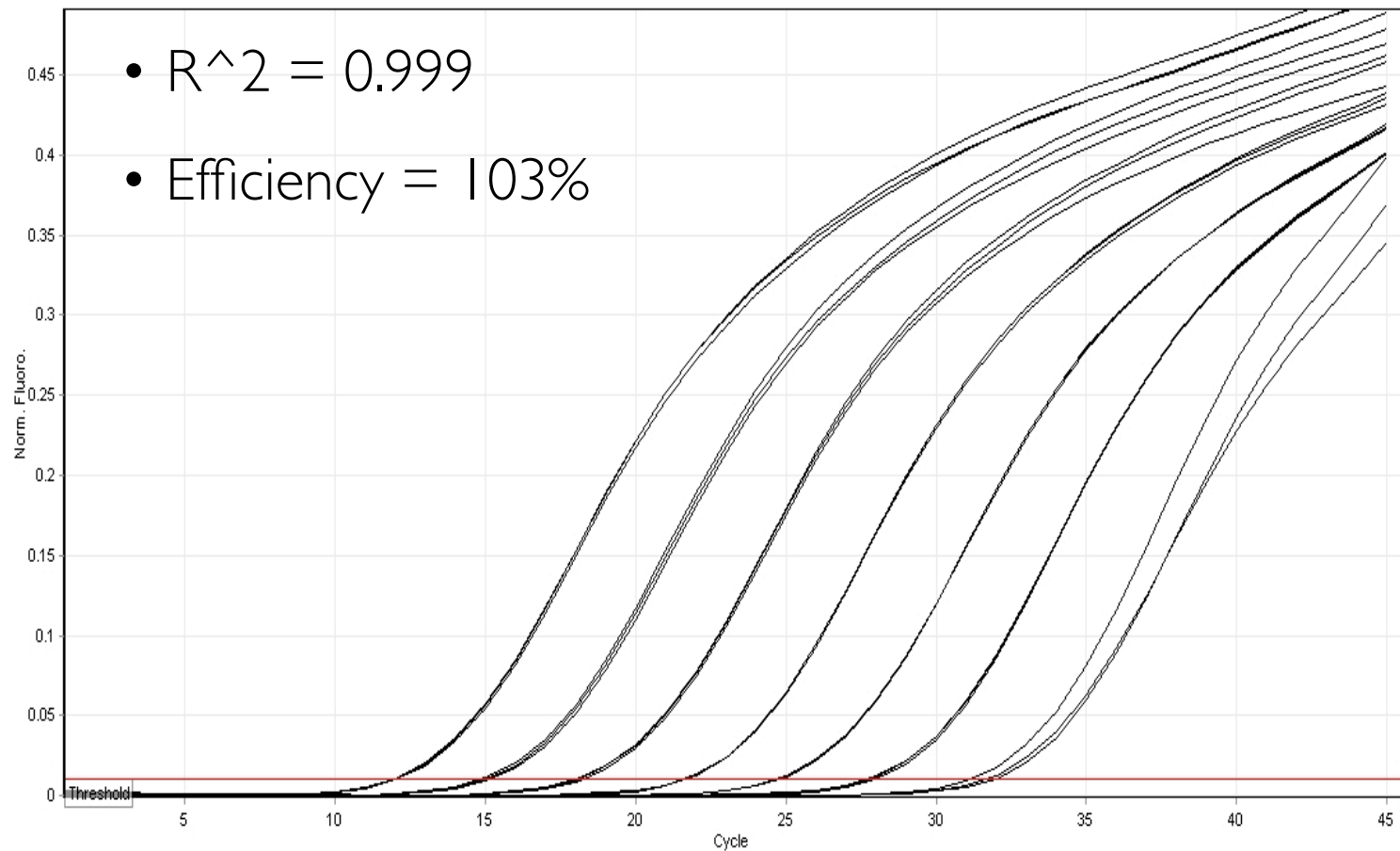
Confirm amplification *in vitro*

Amplification Performance - Gsdma2



Confirm amplification *in vitro*

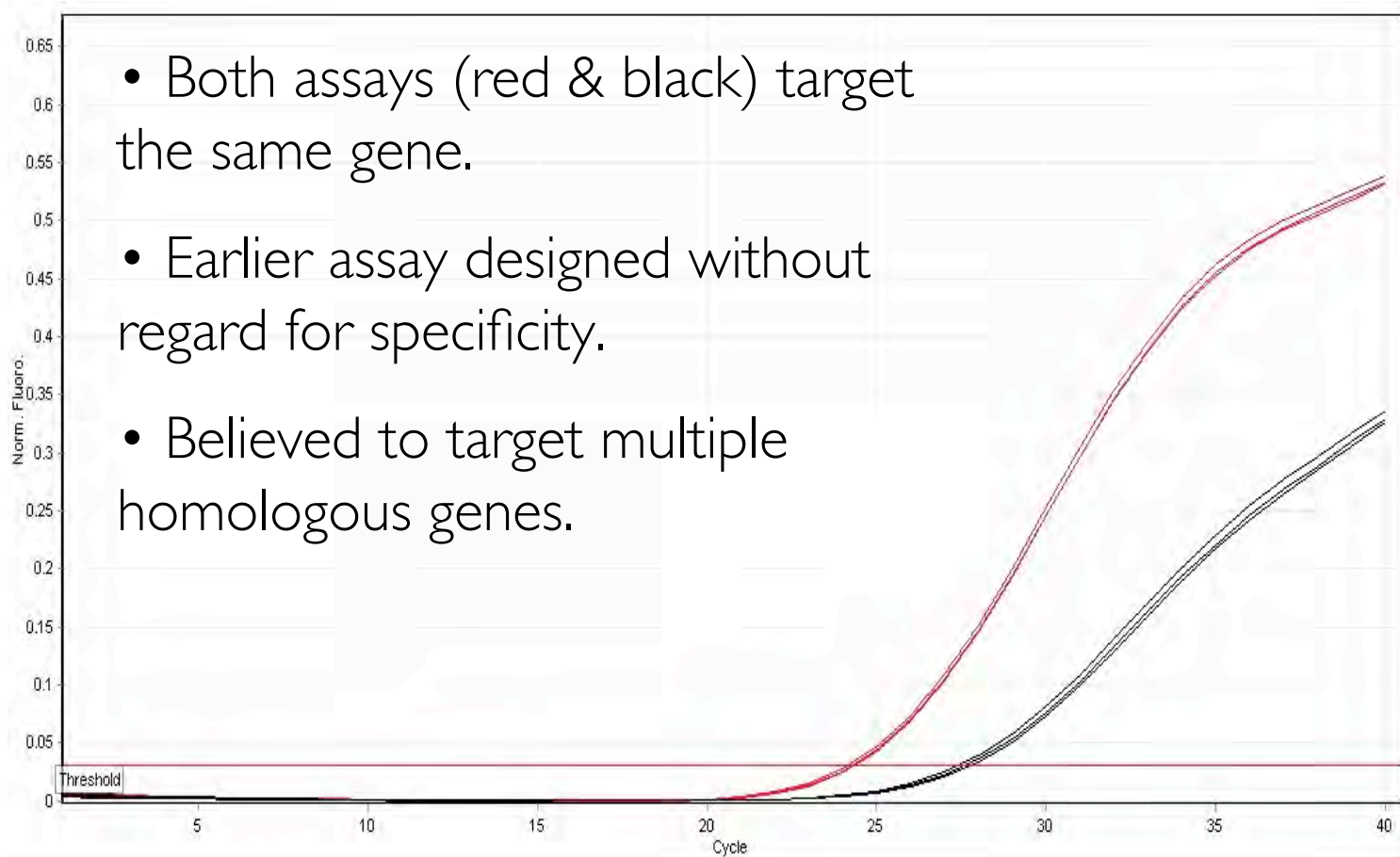
Amplification Performance - Gsdma2



Confirm amplification *in vitro*

Amplification Performance - Bmp8a

- Both assays (red & black) target the same gene.
- Earlier assay designed without regard for specificity.
- Believed to target multiple homologous genes.



Confirm amplification *in vitro*

ACKNOWLEDGEMENTS

Yingzi Xue, Rostislav Chernomorsky, David Frentheway and others in the Velocigene® division of Regeneron.

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